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Neointimal Hyperplasia Following Argon Ion Laser Carotid Endarterectomy: With and Without Endothelial Cell Seeding

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Background and Objective: **This study compares the development of neointimal hyperplasia following conventional and argon ion laser carotid endarterectomy and assesses the potential advantage of endothelial cell seeding.**
Study Design/Materials and Methods: **Eight dogs underwent conventional endarterectomy in one carotid artery and an argon ion laser endarterectomy in the other. After 42 days, these arteries were harvested and the intimal thicknesses were compared. Six additional dogs underwent bilateral argon ion laser carotid endarterectomy with endothelial cell seeding on one side only. These arteries were harvested after 65 days and their mean intimal thicknesses were compared.**
Results: **At 42 days, the mean intimal thickness in the conventional endarterectomy group was 0.070±0.007 mm; in the argon ion laser endarterectomy group it was 0.058±0.001 mm (*P* = 0.76, NS). At 65 days, the mean intimal thickness in the group without endothelial cell seeding was 0.125±0.003 mm vs. 0.061±0.001 mm on the seeded side (*P* = 0.043).**
Conclusion: **Argon ion laser carotid endarterectomy results in no more neointimal hyperplasia than conventional endarterectomy. The neointimal hyperplasia is reduced by endothelial cell seeding. Lasers Surg. Med. 20:367–372, 1997.**

Key words: carotid endarterectomy; argon ion laser; endothelial cell seeding; neointimal hyperplasia; laser endarterectomy

INTRODUCTION

Argon ion laser carotid endarterectomy welds intimal flaps and smooth muscle fibers and may prevent cerebral embolization [1]. However, certain methods of laser endarterectomy may result in increased neointimal hyperplasia and reduced patency [2]. Endothelial cell seeding of carotid arteries results in improved patency and less surface thrombogenicity because endothelial cells secrete substances with anticoagulant and antiproliferative properties [3–5]. This study compares the development of neointimal hyperplasia following conventional and argon ion laser carotid endarterectomy and assesses the potential advantage of endothelial cell seeding in reducing neointimal hyperplasia.

MATERIALS AND METHODS

Fourteen mongrel dogs (weighing 20–30 kg) were used in this study. Each dog was operated on and cared for according to the *Principles of Laboratory Animal Care* formulated by the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and pub-

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lished by the National Institutes of Health. They were housed in our animal care facility and had free access to food and water. The dogs were divided into two groups.

Previous canine studies of neointimal hyperplasia and endothelial cell seeding have used 5–6 dogs per group. This study uses 6–8 dogs per group.

An argon ion laser (HGM Medical Lasers, Salt Lake City, UT, model number 20S) was used for the experiments, with wavelength set at 488 nm.

Group 1

Eight dogs were included in group one. Under intravenous anesthesia using pentobarbital (25 mg/kg) and sterile technique, both carotid arteries were exposed through a midline neck incision. After heparin administration (100 units/kg), the common carotid arteries were clamped proximally and distally and a 4-cm-long arteriotomy was made in each artery.

In one carotid artery, a 1-cm-long argon ion laser endarterectomy was made using a 300 m quartz fiber to deliver laser power set at 1.0 watt, measured at the laser head. The fiber tip was positioned ~ 1 cm away from the working surface during endarterectomy. The argon ion laser carotid endarterectomies were each completed in < 5 minutes. Parallel incisions were made through the intima in a transverse direction, the intima was retracted, and the laser beam was directed at the cleavage plane just beneath the internal elastic lamina. This allowed removal of a 1 cm circumferential segment of intima. The arteriotomy was closed with a running 6-0 polypropylene suture.

In the contralateral carotid artery, a 1-cm-long conventional endarterectomy was made. Parallel incisions were made through the intima in a transverse direction using a 15-blade knife and the intima was mechanically removed, again resulting in removal of a 1 cm circumferential segment of intima. The arteriotomy was closed with a running 6-0 polypropylene suture.

The side (right or left) of laser endarterectomy and conventional endarterectomy was alternated to avoid "side bias."

The animals were sacrificed 6 weeks postoperatively. Immediately prior to sacrifice, the carotid arteries were harvested through the previous neck incision and fixed in formalin solution. Each vessel was imbedded in paraffin, sectioned at 0.6 mm thickness, and stained with trichrome stain. The neointimal thickness in each endarterectomy segment was measured with a micrometer at 12 points around the circumference of the vessel, and a mean neointimal thickness was determined.

Group 2

Six dogs were included in group two. Under intravenous anesthesia using pentobarbital (25 mg/kg) and sterile technique, the external jugular veins were removed through bilateral longitudinal incisions. The endothelial cells from each external jugular vein were harvested and cultured using a previously described technique [3]. After the cultured cells had grown to confluence, they were harvested and resuspended in culture medium to a concentration of 10^6 cells/ml. This concentration is known to result in high density seeding of endarterectomy segments [3].

Both carotid arteries of the dog from whom the endothelial cells were obtained were exposed through a midline incision under intravenous pentobarbital anesthesia. After heparin administration (100 units/kg), the common carotid arteries were clamped proximally and distally, and a 4-cm-long arteriotomy was made in each artery.

In both carotid arteries, a 1-cm-long argon ion laser endarterectomy was made using a 300 m quartz fiber to deliver laser power set at 1.0 watt. The fiber tip was positioned ~ 1 cm away from the working surface during endarterectomy. The argon ion laser carotid endarterectomies were each completed in < 5 minutes. Parallel incisions were made through the intima in a transverse direction, the intima was retracted, and the laser beam was directed at the cleavage plane just beneath the internal elastic lamina. This allowed removal of a 1 cm circumferential segment of intima.

The carotid arteries were held open using atraumatic plexiglass holders. One artery was covered with the endothelial cell suspension for 1 hour, whereas the other artery was covered with culture medium. The arteriotomies were then closed with running 6-0 polypropylene suture.

The animals were sacrificed 65 days postoperatively. Immediately prior to sacrifice, the carotid arteries were harvested through the previous neck incision and fixed in formalin solution. The arteries were imbedded in plastic, sectioned at 0.6 mm thickness, and stained with hematoxylin/eosin stain. The neointimal thickness in each endarterectomy segment was measured with a micrometer at 12 points around the circumference

TABLE 1. Vessel Patency After Conventional and Laser Endarterectomy*

	Patent	Occluded
Conventional	5	3
Laser	6	2

*$P = 0.359$, not significant.

of the vessel, and a mean neointimal thickness was determined.

Although the experiments with groups one and two were initially conducted as two separate studies, they are reported together for completeness. Comparisons were made between animals within the same group. No comparisons were made between animals in different groups.

In group 1, the mean intimal thickness was compared between the conventional and laser endarterectomy segments using the t-test. The patency rates between the conventional and laser endarterectomy segments were compared using a Fisher's exact test.

In group 2, the mean intimal thickness was compared between the unseeded and seeded arteries using the t-test.

RESULTS

Group 1

Of the 16 vessels from eight dogs in group 1, five were occluded. Two laser endarterectomy vessels were occluded, and three conventional endarterectomy vessels were occluded (Table 1). The difference in patency rates between conventional and argon ion laser endarterectomy was not significant. The neointimal thickness of occluded vessels could not be determined and is not included in the comparison of neointimal thickness.

Neointimal thickness of each artery was measured by microscopic examination. The neointimal thickness was readily determined using the trichrome stain, as the fibrous neointimal layer stained bright blue and the muscularis layer stained black (Fig. 1). The neointimal thickness of the argon ion laser endarterectomy specimens ranged from 0.005 mm to 0.093 mm, with a mean thickness of 0.058±0.001 mm. The neointimal thickness of the conventional endarterectomy specimens ranged from 0.01 mm to 0.2 mm, with a mean thickness of 0.070±0.007 mm (Fig. 2). The difference is not statistically significant ($P = 0.76$).

Group 2

None of the vessels in group 2 were occluded. The neointimal thickness of each artery was measured by microscopic examination. The neointimal thickness was more difficult to determine with H&E stain because the difference between the neointima and the media was not as readily apparent as in the arteries stained with trichrome stain (Fig. 3). The neointimal thickness of the seeded arteries ranged from 0.03 mm to 0.10 mm, with a mean thickness of 0.061±0.001 mm (Fig. 4). The neointimal thickness of the unseeded arteries ranged from 0.065 mm to 0.228 mm, with a mean thickness of 0.125±0.003 mm. The difference is statistically significant ($P = 0.043$).

DISCUSSION

Argon ion laser carotid endarterectomy offers the advantage of a smooth vessel surface [4]. It can securely weld intimal flaps and smooth muscle fibers and may prevent embolization [2,4]. However, exposure of arterial walls to laser energy may increase the rate of acute thrombosis and the later development of neointimal hyperplasia. This is the first study to compare directly the amount of noeintimal hyperplasia following conventional and laser carotid endarterectomy and the long-term effect of endothelial cell seeding following argon ion laser carotid endarterectomy.

Clinical reports of the use of argon ion laser carotid endarterectomy have shown successful outcome in a small number of patients [5]. Following conventional carotid endarterectomy, there is a 4–15% incidence of carotid restenosis due to neointimal hyperplasia [6]. There is no reported incidence of carotid restenosis following argon ion laser endarterectomy.

Cikrit et al. [2] reported an increased amount of neointimal hyperplasia following exposure of denuded arteries to laser energy. They performed conventional endarterectomy followed by g-lasing of the denuded surface with the CO_2 laser. The clinical reports of argon ion laser carotid endarterectomy and this study, however, used laser energy to separate the intima from the underlying vessel at the cleavage plane developed during endarterectomy. We found no difference in the amount of neointimal hyperplasia following argon ion laser and conventional endarterectomy, or in the thrombogenicity of the denuded surface.

The causes of neointimal hyperplasia after

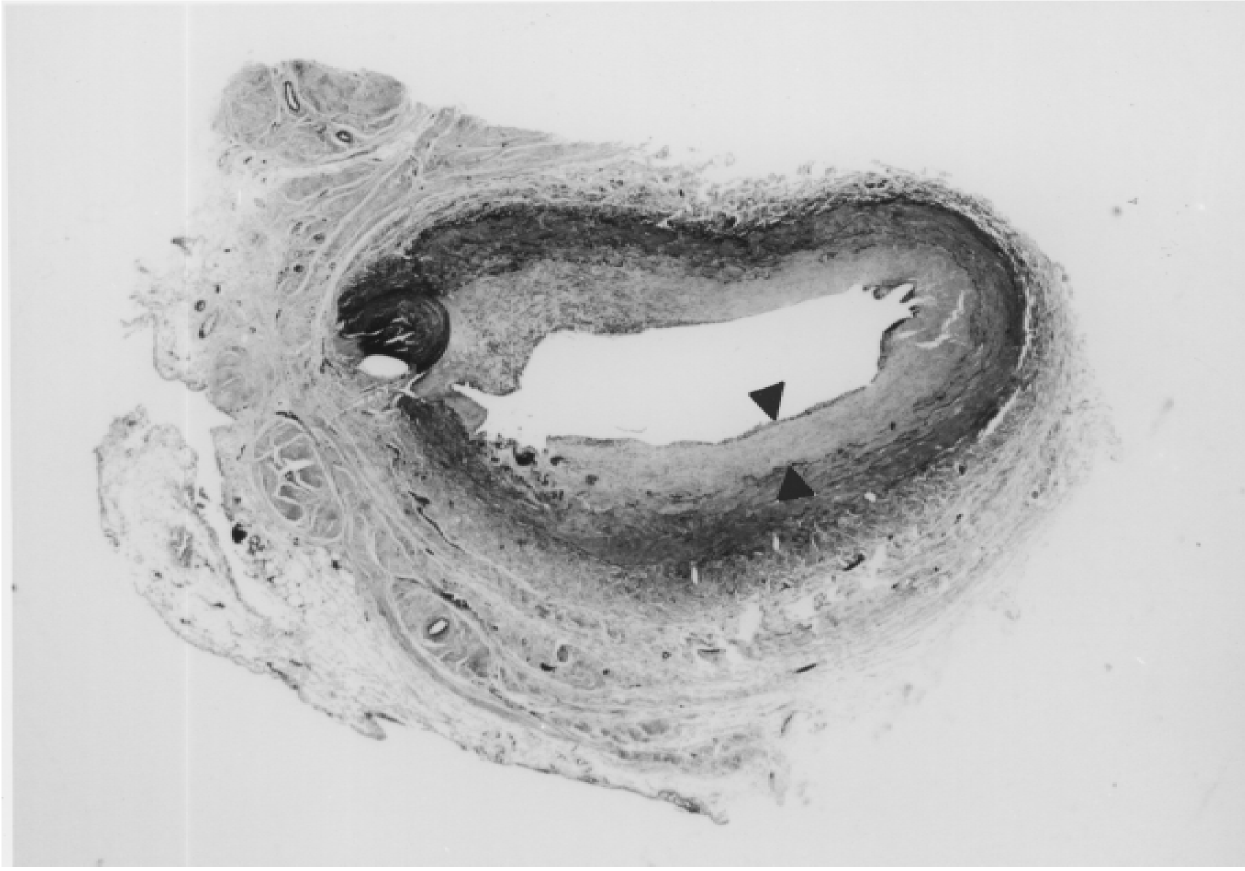

Fig. 1. Photomicrograph of carotid artery after argon ion laser endarterectomy. Arrowheads demonstrate neointimal thickness. Trichrome stain (magnification ×4).

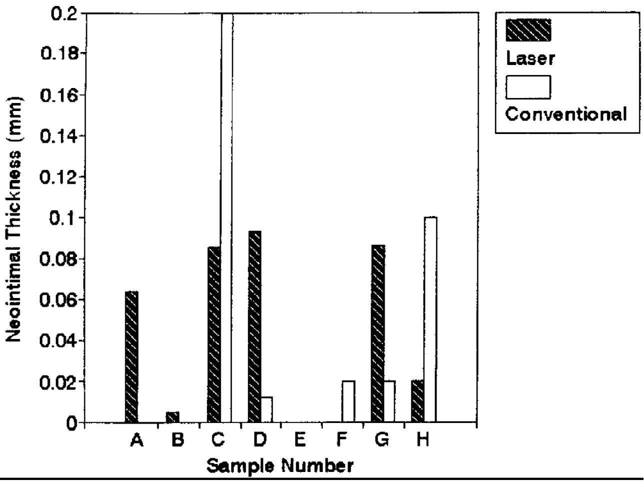

Fig. 2. Neointimal hyperplasia following laser and conventional endarterectomy.

endarterectomy are not completely clear. Platelet-vessel wall interaction has been implicated in the process], as well as exposure of the vessel wall to leukocytes. After endarterectomy, platelets adhere to the denuded surface and release vasoactive and growth factor substances, including thromboxane and platelet derived growth factor [7]. Smooth muscle fibers are then presumably stimulated to migrate to the luminal surface and proliferate. Connective tissue is also formed in the neointima, stimulated by unidentified factors possibly released by the smooth muscle fibers [8]. Our finding in this canine model that there is no difference in neointimal hyperplasia following conventional and laser endarterectomy is not surprising if one considers that the result of *both* processes is a surface denuded of endothelium, with exposed collagen, to which platelets adhere and presumably stimulate smooth muscle growth.

Platelet aggregation and adhesion to denuded vessel wall are probably important first steps in the formation of neointimal hyperplasia. Aspirin does not inhibit the formation of neointimal hyperplasia in experimental models because it does not inhibit the adherence of platelets to collagen [11]. Endothelial cell seeding has been

Fig. 3. Photomicrograph of carotid artery following argon ion laser endarterectomy and endothelial cell seeding. Arrowheads indicate neointimal thickness. Open arrow points to endothelial cell. H&E stain (magnification ×10).

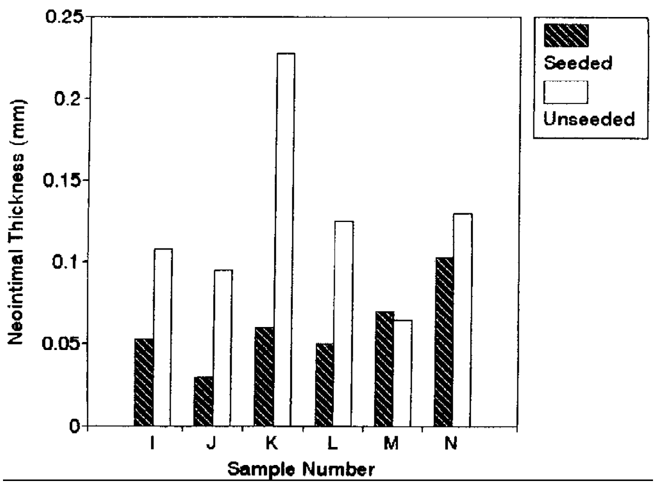

Fig. 4. Neointimal hyperplasia in carotid arteries subjected to argon ion laser endarterectomy, with and without endothelial cell seeding.

shown experimentally to decrease the amount of neointimal hyperplasia after conventional carotid endarterectomy [11]. The reason for this observed decrease is not entirely clear. The endothelium on the surface of the vessel may block the adherence of platelets and their subsequent action on smooth muscle fibers. In addition, seeded endothelium on the surface of the vessel inhibits smooth muscle cell growth. These two factors have been demonstrated by Bush et al. [12]. They showed that endothelial cell seeding after endarterectomy results in higher intraluminal concentrations of prostacyclin, less platelet adherence, and lower thromboxane concentrations than unseeded vessels subjected to conventional endarterectomy. Additionally, endothelium secretes a heparin-like compound that inhibits smooth muscle cell growth [13].

Cultured endothelium can be seeded in adequate numbers on the carotid artery surface following argon ion laser carotid endarterectomy [3]. Seeding has been shown in this model to decrease the acute thrombosis rate and to decrease the number of platelets adherent to the arterial surface following endarterectomy [3]. Other investigators

have found a significant reduction in neointimal hyperplasia following conventional endarterectomy after endothelial cell seeding [13]. We have shown the same effect following argon ion laser carotid endarterectomy.

Argon ion laser carotid endarterectomy has been used clinically with good results. We have shown in the experimental model that the technical advantages of laser endarterectomy do not come at the expense of long-term patency. Given its advantages, argon ion laser carotid endarterectomy should continue to be used clinically and may offer better results than conventional endarterectomy.

From this study and previous studies by the authors and others [11–13], endothelial cell seeding of carotid arteries following endarterectomy (both conventional and laser endarterectomy) improves healing, decreases acute thrombosis, and decreases neointimal hyperplasia. Continued clinical trials of laser endarterectomy and endothelial cell seeding are warranted.

ACKNOWLEDGMENTS

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